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OFFICE OF  
PREVENTION, PESTICIDES AND  
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## MEMORANDUM

**SUBJECT:** Mutagenicity Hazard Review of P08-508 and -509

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*Michael C. Cimino*  
8/29/2008

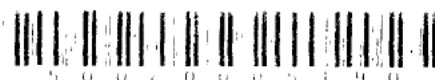
**TO:** Steven Cragg, PhD, DABT  
Technical Integrator  
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**THRU:** *[Signature]* Donald Rodier *[Signature]* 9/5/08  
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## I. CONCLUSION

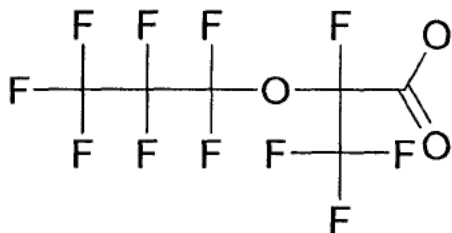
Based on data on the PMNs themselves and on analogues, P08-508 and P08-509 are or may be: (a) not gene mutagens in two species of prokaryotes; (b) chromosome mutagens in mammalian and human cells in culture, but not in mammals *in vivo* and; (c) not inducers of DNA effects in mammalian cells *in vivo*.

The positive data on the PMN for *in vitro* chromosomal aberrations in mammalian and human cells are of some concern. However, the negative responses for *in vivo* chromosomal

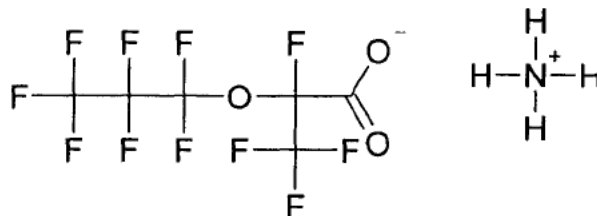


effects as micronuclei and as chromosomal aberrations, and for induction of DNA effects, alleviate that concern. There is no basis for recommending additional mutagenicity testing for the PMNs, and there is little support for a cancer concern based upon mutagenicity. The lack of mutagenicity concern does not negate a cancer concern should such concern be based upon nongenotoxic information.

## II. STRUCTURES OF P08-508, -509 AND ANALOGUES



P08-508



P08-509




### III. BASIS FOR THE CONCLUSIONS

Mutagenicity data were provided with the Premanufacturing Notice on P08-508 and -509:

A) P08-508 is Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-;

 CAS# 13252-13-6

B) P08-509 is Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt (1:1);  CAS# 62037-80-3

and on three transformation products:



The structures of these chemicals are provided in Section II. The data are reviewed below.

## A) P08-508

### I. Bacterial reverse mutation

P08-508 was tested in a bacterial reverse mutation assay, as reported in "[REDACTED]: Bacterial reverse mutation test", conducted by [REDACTED] study (Report Revision 1) dated February 22, 2008 (PMN Attachment 79). The PMN [REDACTED] was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *Escherichia coli* strain WP2uvrA, both without and with metabolic activation using Aroclor 1254-induced rat liver S9. It was tested at eight dose levels ranging from 33.3 to 5,000 µg/plate, in duplicate plates per dose level. Dose selection was acceptable for a noncytotoxic chemical. An independent repeat was conducted, in triplicate plates, at five dose levels from 333 to 5,000 µg/plate. The chemical did not induce significant increases in gene mutations under any test condition. Concurrent negative (the solvent, sterile water) and positive controls produced appropriate responses.

### II. *In vitro* chromosome aberration

The PMN also was tested in an *in vitro* mammalian chromosome aberration assay, as reported in "[REDACTED] *In vitro* mammalian chromosomal aberration test in Chinese hamster ovary cells," also conducted by [REDACTED], study (Report Revision 1) dated February 25, 2008 (PMN Attachment 78). The PMN (purity as above) was tested both without and with metabolic activation using rat liver S9, as above. Dose selection was based upon the results of a Preliminary Toxicity Assay. The dose level of 3471 µg/ml, which represented 10mM, was the limit dose for this chemical in this assay. Two experiments were conducted, in duplicate flasks for each treatment group. The first experiment involved exposure of the cells for four hours with 16h recovery, conducted without and with activation. Five dose levels of 100, 500, 1,000, 2,000 and 3,471 µg/ml were applied. The highest three doses were used for mutagenicity evaluation. The second experiment involved exposure for 20h with no recovery period, and was conducted without activation only. The same five dose levels were applied as for the first experiment. Cytotoxicity (>50%) was noted only at the highest dose in the 20hr treatment. Thus the three highest surviving doses used for mutagenicity evaluation in the second experiment were 100, 500 and 1,000 µg/ml. Dose selection was acceptable. No significant increases in structural aberrations were detected under any treatment condition. Numerical aberrations (polyploidy) were increased in a statistically-significant manner, with dose responses, in the first (4h) experiment both without and with activation. Concurrent negative (sterile water) and positive controls (mitomycin C and cyclophosphamide for non-activated and activated assays, respectively) produced appropriate responses.

In summary, P08-508 is not a gene mutagen in two species of prokaryotes both without and with activation. It induces chromosomal mutations in mammalian cells *in vitro* in the form of numerical but not structural aberrations both without and with activation.

## B) P08-509

### I. Bacterial reverse mutation

P08-509 was tested in a bacterial reverse mutation assay, as reported in "[REDACTED]; Bacterial reverse mutation test", conducted by [REDACTED] study dated July 26, 2007 (PMN Attachment 68). The PMN [REDACTED] was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and in *Escherichia coli* strain WP2uvrA, both without and with metabolic activation using Aroclor 1254-induced rat liver S9. It was tested at eight dose levels ranging from 33.3 to 5,000 µg/plate, in duplicate plates per dose level. Dose selection was acceptable for a noncytotoxic chemical. An independent repeat was conducted, in triplicate plates, at five dose levels from 333 to 5,000 µg/plate. The chemical did not induce significant increases in gene mutations under any test condition. Concurrent negative (the solvent, sterile water) and positive controls produced appropriate responses.

### II. *In vitro* chromosome aberration

The PMN also was tested in an *in vitro* mammalian chromosome aberration assay, as reported in "[REDACTED] *In vitro* mammalian chromosomal aberration test in Chinese hamster ovary cells," also conducted by [REDACTED] study dated July 25, 2007 (PMN Attachment 69). The PMN (purity as above) was tested both without and with metabolic activation using rat liver S9, as above. Dose selection was based upon the results of a Preliminary Toxicity Assay. The dose level of 3471 µg/ml, which represented 10mM, was the limit dose for this chemical in this assay. Two experiments were conducted. The first experiment involved exposure of the cells for four hours with 16h recovery, and was conducted without and with activation. Three dose levels of 1,000, 2,000 and 3,471 µg/ml were evaluated. The second experiment involved exposure for 20h with no recovery period, and was conducted without activation only. Three dose levels of 500, 1,000 and 2,000 µg/ml were evaluated. Cytotoxicity (>50%) was noted only at the highest dose in the 20hr treatment without activation. Dose selection was acceptable. Statistically significant increases in structural aberrations were detected for the 4h experiment with activation. No significant increases in structural aberrations were observed without activation, or in the 20h experiment either without or with activation, or for numerical aberrations under any test condition. Concurrent negative (sterile water) and positive controls (mitomycin C and cyclophosphamide for non-activated and activated assays, respectively) produced appropriate responses.

### III. The *in vivo* micronucleus and chromosome aberration study

The PMN was tested for induction *in vivo* of micronuclei and chromosome aberrations, as reported in [REDACTED]: "In vivo micronucleus and chromosome aberrations assay in mouse bone marrow cells". The study was conducted by [REDACTED] study dated 10 October 2007 (PMN Attachment 92). The PMN (purity as above) was tested in male and female ICR mice. In a range-finding study, 4 of 5 male and 4 of 5 females died at 2,000 mg/kg body weight; in a subsequent study 1,300 mg/kg was determined to be the maximum tolerated dose. In the mutagenicity test proper, animals (5 males and 5 females per dose group) were exposed to three dose levels of 325, 650 and 1,300 mg/kg by a single oral administration (PO) and sacrificed 24 hours after exposure. Dose selection was acceptable. No significant increases in micronucleated polychromatic erythrocytes (mPCEs) or in chromosomal aberrations, either structural or numerical, were observed for any test condition. Concurrent negative (sterile water) and positive (cyclophosphamide) controls produced appropriate responses.

### IV. *In vivo* Unscheduled DNA Synthesis (UDS)

The PMN also was tested for *in vivo* DNA effects in a UDS assay, as reported in [REDACTED]: "Unscheduled DNA synthesis (UDS) test with mammalian cells *in vivo*", also conducted by [REDACTED], study dated 14 August 2007 (PMN Attachment 71). The PMN (purity as above) was tested in male rats exposed by oral gavage. Animals (5 males per dose group) were exposed to 500, 1,000 and 2,000 mg/kg body weight for 5 days/week, for 4 weeks, and harvest of animals occurred at 2-4h and at 12-16h after termination of exposure. Dose levels were based upon an initial rangefinding study, and were acceptable. No mortality was observed in the assay. No significant increases in net nuclear grain counts were observed. Concurrent negative (distilled water) and positive controls (dimethylnitrosamine) produced appropriate responses.

In summary, P08-509 is not a gene mutagen in two species of prokaryotes both without and with activation. It induces chromosomal mutations in mammalian cells *in vitro* in the form of structural aberrations with activation but not without, and it does not induce numerical aberrations both without and with activation. It does not induce chromosomal mutations in mammals *in vivo* by the oral route in the form of structural aberrations, numerical aberrations, or micronuclei, nor DNA effects in the form of unscheduled DNA synthesis.

## C) [REDACTED]

### I. Bacterial reverse mutation

The test material was tested in a partial bacterial reverse mutation assay, as reported in "Mutagenicity testing of [REDACTED] in the *Salmonella typhimurium* plate incorporation test", conducted by [REDACTED], study dated November 10, 1994

(PMN Attachment 117). The PMN [REDACTED] was tested in *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535, both without and with metabolic activation using Aroclor 1254-induced rat liver S9. Since the study included only four bacterial strains instead of the usual five, it does not meet OECD or OPPT guideline requirements for the bacterial reverse mutation test. It was tested at seven dose levels ranging from 10.0 to 5,000.0 µg/plate, in triplicate plates per dose level. Dose selection was acceptable for a noncytotoxic chemical. There was no confirmatory study. The chemical did not induce significant increases in gene mutations under any test condition. Concurrent negative (the solvent, acetone) and positive controls produced appropriate responses.

## II. *In vivo* micronucleus assay

The test material was tested in the *in vivo* micronucleus assay, as reported in "Combined two-week inhalation toxicity and micronucleus studies with [REDACTED] and [REDACTED] in rats". The study was conducted by [REDACTED] study dated September 21, 1995 (PMN Attachment 118). The chemical [REDACTED] was tested in male and female Crl:CD BR rats exposed by inhalation for 6h/day, 5 days/week for 2 weeks, to 5,000, 25,000 and 175,000 ppm of [REDACTED]. No systemic toxicity and no effect on bone marrow as defined by depression of the ratio of polychromatic to normochromatic erythrocytes were observed in the study. Dose selection was acceptable for a gaseous chemical. No significant increases in micronucleated polychromatic erythrocytes (mPCEs) were observed for any test condition. Concurrent negative (air) and positive controls (cyclophosphamide administered in a single intraperitoneal injection) produced appropriate responses.

In summary, the analogue [REDACTED] is not a gene mutagen in one species of prokaryote both without and with activation, although this study is considered incomplete. It does not induce chromosomal mutations in mammals *in vivo* by the inhalation route in the form of micronuclei.

## D) [REDACTED]

### I. *In vivo* micronucleus assay

The test material was tested in the *in vivo* micronucleus assay, as reported in "Combined two-week inhalation toxicity and micronucleus studies with [REDACTED] and [REDACTED] in rats". This is the same study as for chemical C above (PMN Attachment 118). The chemical [REDACTED] was tested in male and female Crl:CD BR rats by inhalation for 6h/day, 5 days/week for 2 weeks, to one dose level, 25,000 ppm. The adequacy of the dose level cannot be determined since no systemic toxicity and/or no effect on bone marrow as defined by depression of the ratio of polychromatic to normochromatic erythrocytes was observed in the study. No significant increases in micronucleated polychromatic erythrocytes (mPCEs) were observed for any test

condition, but these results are of uncertain value due to the question about the adequacy of the dose selection. Concurrent negative (air) and positive (cyclophosphamide) controls produced appropriate responses

In summary, the analogue [REDACTED] does not induce chromosomal mutations in mammals *in vivo* by the inhalation route in the form of micronuclei, but this study is considered inconclusive due to uncertainty about the adequacy of the dose tested.

E) [REDACTED] 1

I. *In vitro* chromosome aberration

The test material was tested in an *in vitro* mammalian chromosome aberration assay, as reported in "In vitro evaluation of [REDACTED] for chromosome aberrations in human lymphocytes," conducted by [REDACTED], study dated December 19, 1994 (PMN Attachment 116). The test material was identified as "[REDACTED]", and was comprised of two components: [REDACTED]

It was tested both without and with metabolic activation using rat liver S9. Two experiments were conducted. The first experiment involved exposure of the cells for 3 hours, and harvest of cells 18-20 hours after the end of treatment. Five dose levels of 0.3, 1.0, 1.9, 2.9, 3.8 and 5.1 mg/ml (equivalent to 300, 1,000, 1,900, 2,900, 3,800 and 5,100 µg/ml) were applied. The highest four doses were used for mutagenicity evaluation. The second experiment was the same as the first, with an additional harvest of cells 24 hours after the initial harvest, at the same dose levels as for the first experiment. Again the four highest surviving doses were used for mutagenicity evaluation. Dose selection was acceptable. Without activation, there were statistically significant increases in structural aberrations at the two highest doses in the first experiment and at the second highest dose (3.8 mg/ml) in the second experiment; with activation there were statistically significant increases at 1.9 and 2.9 mg/ml in the first experiment and at 1.9 and 3.8 mg/ml in the second experiment. Cells from the additional harvest time for the second experiment were not evaluated since positive responses had already been obtained for the earlier harvests. Concurrent negative (acetone) and positive controls (mitomycin C and cyclophosphamide for non-activated and activated assays, respectively) produced appropriate responses.

In summary, the analogue [REDACTED] induces chromosomal mutations in human cells *in vitro* in the form of structural aberrations both without and with activation.

There are mutagenicity data available on analogues of the PMNs. These have been recently reviewed for [REDACTED]. The structures for these analogues are also in Section II. The mutagenicity data are summarized as follows:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

The data on PMNs 08-508 and -509, its three transformation products, and four other analogues, are summarized in the Table on the next page:

TABLE: MUTAGENICITY DATA ON P08-508, -509 AND ANALOGUES

Chemical	Gene mutations		Chromosome mutation				DNA
	SAL	E.coli	cytogenetics mam vt		MN vv	CA vv	UDS
			CA struc	CA num			
P08-508	neg wo & w	neg wo & w	neg CHO wo & w	pos CHO wo & w			
P08-509	neg wo & w	neg wo & w	pos CHO w & neg wo	neg CHO w & wo	neg m&f mou PO	neg m&f mou PO	neg UDS m rat PO
██████	inc neg wo & w				neg MN m rat inh		
██████					inc neg MN m rat inh		
██████			pos hum lymph wo & w				
██████	neg wo & w	neg wo & w	pos CHL wo & w				
██████	neg wo & w	neg wo & w					
██████	neg wo & w	neg wo & w					
██████	neg wo & w	neg wo & w					

Abbreviations used in Table:

CA = chromosomal aberrations; CHL = Chinese hamster lung fibroblasts; CHO = Chinese hamster ovary cells; E.coli = *Escherichia coli*; f = female; hum lymph = human lymphocytes; inc = inconclusive; inh = inhalation m = male; mam = mammalian cells; MN = micronuclei; mou = mouse; neg = negative; num = numerical aberrations; PO = oral gavage; pos = positive; SAL = *Salmonella*; struc = structural aberrations; UDS = unscheduled DNA synthesis; vt = *in vitro*; vv = *in vivo*; w = with activation; wo = without activation

Thus, based upon data on the PMNs themselves and on analogues, P08-508 and P08-509 are or may be: (a) not gene mutagens in two species of prokaryotes; (b) chromosome mutagens in mammalian and human cells in culture, but not in mammals *in vivo* and; (c) not inducers of DNA effects in mammalian cells *in vivo*.

The positive responses for chromosome aberrations *in vitro* for polyploidy in CHO cells (█-508), for structural aberrations in CHO cells (P08-509), for structural aberrations in human cells (█), and for aberrations in CHL cells (█), are of some concern. However, the negative responses in *in vivo* chromosomal assays for micronuclei and for chromosomal aberrations PO (P08-509), and for micronuclei by inhalation (█), alleviate the concern. In addition, there is negative evidence that P08-509 induces DNA effects (█) *in vivo*.

Due to these *in vivo* results, there is no basis for recommending additional mutagenicity testing for the PMNs █-508 and -509, and there is little support for a cancer concern based upon mutagenicity. The negative mutagenicity concern does not negate a cancer concern should such concern be based upon nongenotoxic information.

#### IV. REFERENCE

Cimino, MC. 2008. U. S. Environmental Protection Agency, Office of Pollution Prevention and Toxics. Mutagenicity Hazard Review of █. Intradivision memorandum to S Cragg, dated January 18, 2008. USEPA. Washington, DC 20460.